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Review

Association between *ACTN3* R577X genotype and risk of non-contact injury in trained athletes: A systematic review

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Abstract

Background: The aim of this study was to review, systematically, evidence concerning the link between the *ACTN3* R577X polymorphism and the rates and severity of non-contact injuries and exercise-induced muscle damage in athletes and individuals enrolled in exercise training programs.

Methods: A computerized literature search was performed in the electronic databases PubMed, Web of Science, and SPORTDiscus, from inception until November 2020. All included studies compared the epidemiological characteristics of non-contact injury between the different genotypes of the *ACTN3* R577X polymorphism.

Results: Our search identified 492 records. After the screening of titles, abstracts, and full texts, 13 studies examining the association between the *ACTN3* genotypes and the rate and severity of non-contact injury were included in the analysis. These studies were performed in 6 different countries (Spain, Japan, Brazil, China, Republic of Korea, and Italy) and involved a total participant pool of 1093 participants. Of the studies, 2 involved only women, 5 involved only men, and 6 involved both men and women. All the studies included were classified as high-quality studies (≥6 points on the Physiotherapy Evidence Database [PEDro] scale). Overall, evidence suggests there is an association between the *ACTN3* R577X genotype and non-contact injury in 12 investigations. Six studies observed a significant association between *ACTN3* R577X polymorphism and exercise induced muscle damage: 2 with non-contact ankle injury, 3 with non-contact muscle injury, and 1 with overall non-contact injury.

Conclusion: The present findings support the premise that possessing the *ACTN3* XX genotype may predispose athletes to a higher probability of some non-contact injuries, such as muscle injury, ankle sprains, and higher levels of exercise-induced muscle damage.

Keywords: α-actinin-3 deficiency; Athletic performance; Exercise-related injury; Muscle injury; Single nucleotide polymorphism

1. Introduction

The protein α-actinin-3 is a structural component of the Z-disc encoded by the *ACTN3* gene. The main role of α-actinin-3 is anchoring actin thin filaments to assist in maintaining the myofibrillar array, which contributes to regulating muscle length and tension during contraction.[1] Interestingly, α-actinin-3 is expressed only in fast muscle fibers (all fast-glycolytic type 2X fibers and 50% of fast oxidative type 2A fibers),[2] suggesting a specific function for powerful and fast muscle contractions. A common genetic variant in the *ACTN3* (rs1815739, also known as the R577X polymorphism) leads to the replacement of an arginine (R) with a premature stop codon (X) at amino acid 577. Consequently, individuals with

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the *ACTN3* XX genotype are α-actinin-3 protein deficient due to the lack of protein expression. In contrast, homozygous individuals for the R allele (RR genotype) or heterozygote individuals (RX genotype) express α-actinin-3, although recently it has been found that the capacity to express α-actinin-3 is dose-dependent for RR *vs*. RX individuals.[3] The deficiency of α-actinin-3 does not entail any disease or clinical condition but has been demonstrated to produce some potentially negative phenotypes in humans, such as lower muscle strength,[4,5] reduced muscle volume,[6,7] impaired capacity to tolerate the strain produced by explosive muscular actions,[8] and/or an association with decreased bone mineral density.[9]

In athletes, several investigations have confirmed that α-actinin-3 deficiency due to the *ACTN3* XX genotype may negatively influence sprint and power performance.[10] Lower performance in sport disciplines requiring near-to-maximal production of strength or power has been associated with decreased capacity of the ability of muscle fibers to produce powerful contractions in the absence of α-actinin-3.[7] This notion is supported by several investigations that found a higher occurrence of the *ACTN3* RR variant in elite sprint/power athletes when compared with nonathletes.[1] In contrast, a higher-than-expected frequency of the *ACTN3* XX genotype has been found in some groups of elite endurance athletes,[9,11] although the overrepresentation of the XX genotype has not been replicated in other cohorts of elite athletes.[12,13] Collectively, the information obtained from studies investigating the frequency of the different *ACTN3* genotypes in cohorts of elite athletes points toward a negative influence of the XX genotype on elite sprint/power-based exercise (hence, a positive influence of the R allele on this type of exercise) with little or no effect of the XX genotype on endurance-based exercise.

In addition to performance, the absence of α-actinin-3 has been linked to a higher probability of injury[14] in several sport and exercise scenarios. For example, soccer (football) players with the *ACTN3* XX genotype suffered a higher incidence of non-contact muscle injuries when compared to players with the RR or the RX genotype.[15,16] A higher probability of muscle-type injuries was also found in XX compared to RR runners.[17] On the other hand, at least 1 investigation has reported that athletes with the RR or the RX genotype had an increased risk of muscle injury when practicing various sport activities.[18] Additionally, there is an increase in passive hamstring stiffness in R-allele individuals compared to XX counterparts,[19] although the link between muscle stiffness and increased muscle injury risk has not yet been established.[14,20] The most consistent specific findings posit a link between the XX genotype and ankle injuries[21–23] and higher levels of exercise-induced muscle damage.[24–26] These associations suggest a negative impact of α-actinin-3 deficiency on muscle and ligament capacity to endure the forces generated during exercise and point toward an increased susceptibility to contraction-induced damage.[8,9] Despite the extant literature indicating an influence of the *ACTN3* genotype on exercise-related non-contact injuries, there is no previous investigation, to our knowledge, that has systematically reviewed the influence of the *ACTN3* R577X polymorphism on non-contact injuries. Hence, the aim of this investigation was to review evidence systematically concerning the link between the *ACTN3* R577X polymorphism and the rates and severity of non-contact injuries and exercise-induced muscle damage in athletes and individuals enrolled in exercise-training programs.

2. Materials and Methods

2.1. Eligibility criteria

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[27] Only studies that examined the link between the *ACTN3* R577X polymorphism and the rate or severity of non-contact injuries in individuals who were at any level/sex/age and currently participating in a sport or exercise-training program were included. The following inclusion criteria were established for studies of this topic (Table 1). The studies (1) were available in peer-reviewed journals; (2) involved participants aged ≥14 years; (3) included trained athletes (frequency of training ≥5 times/week) and individuals enrolled in exercise training programs (i.e., nonathletes); (4) used validated methods for the characterization of injury epidemiology (e.g., injury severity, occurrence and incidence); and (5) obtained epidemiological data about non-contact injuries developed during sport competitions and exercise practice. Studies were excluded if they: (1) did not contain an experiment study design with the original data, such as books, systematic or narrative reviews, case studies, or opinion pieces; (2) did not meet the minimum requirements to classify the sample as exercisers (frequency of training <3 times/week); or (3) were not written in English.

Table 1
Inclusion criteria according to the PICOS approach.

Criteria	Inclusion	Exclusion
1	Original article published in peer-reviewed journals	Books, reviews, case studies, opinion pieces, non-peer-reviewed journals
2	Involved professional, amateur, or recreational athletes or individuals practicing exercise in regular programs	Untrained individuals
3	Participants > 14 years old, described as young and/or senior athletes	Participants < 14 years old, described as child athletes
4	Analyzed the effects of the *ACTN3* R577X polymorphism on the rates or severity of non-contact injuries	A gene polymorphism other than *ACTN3* R577X analyzed
5	Used quantification of injuries (e.g., injury severity, occurrence, and incidence)	Rates or severity of non-contact injuries not reported
6	Full text available in English	Full text not accessible in English

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2.2. Literature search strategy

Literature searches were conducted in 3 electronic databases including PubMed, Web of Science, and SPORTDiscus, from inception until November 2020. No year restriction was used for the search strategy, in an effort to obtain all studies on the topic. The following key terms (and synonyms searched for by the MeSH database) were included and combined using the operators "AND," "OR," "NOT": ((Alpha-actinin-3 OR ACTN3) OR ("ACTN3 gene" OR "ACTN3 R577X polymorphism" OR "ACTN3 R577X" OR "ACTN3 R577R" OR "ACTN3 577XX genotype*" OR "ACTN3 577RR genotype*" OR "ACTN3 577RX genotype*") AND ("injuries" OR "injury" OR "muscle injury*" OR "non-contact injury" OR "strain" OR "soreness*" OR "sprain*" OR "contusion" OR "strain" OR "fracture" OR "dislocation" OR "concussion") AND (correlation study OR association OR relationship)). The references used in the identified studies were searched as well, in order to identify additional relevant research papers. The search for published studies was independently performed by 2 authors, and disagreements were resolved through discussion.

2.3. Study selection

The screening and study selection were performed by 2 authors based on the above-mentioned inclusion and exclusion criteria. If the title and abstract of the article showed any potential relevance in terms of assessing the influence of the *ACTN3* genotype on the rate and severity of non-contact injuries, the full text was examined. A third author was consulted if the 2 investigators responsible for the study selection were not able to reach an agreement on the inclusion of an article. Fig. 1 depicts the details of the study-selection methodology.

2.3.1. Data extraction

Once the inclusion/exclusion criteria were applied, data extraction was conducted to collect information about participants, intervention, comparisons, outcomes, and study design (PICOS) following PRISMA methodology. The following relevant data from each study were extracted: study details (author, year of publication, country, duration of follow-up), study population (sample size, age, participants' sex), sport or main exercise activity performed by the participants, the method used to determine the genotyping of *ACTN3* R577X (rs1815739), injury definition, workload (external and internal load parameters), and measures of association (i.e., relative risk [RR] or odds ratio [OR]). Where possible, these associations were extracted directly from the original article. For articles in which this information was not present, associations were calculated using raw data, if available. Load parameters were classified as external or internal based on the International Olympic Committee consensus statement on load in sport and risk of injury.[28]

2.3.2. Quality assessment

The methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) scale.[29] A cut-off point of 6 points in the PEDro scale was used to discriminate high-quality studies, and only studies with scores above this threshold were considered for the systematic review.[26] Two authors independently assigned a score

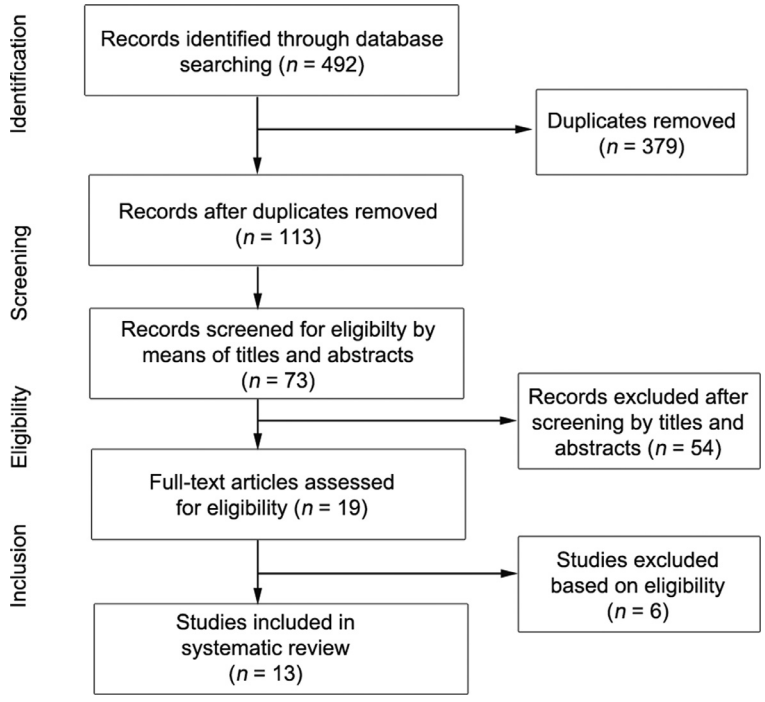

Fig. 1. Selection of research articles ($n = 13$) included in this systematic review using the recommendations in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[26]

to each study using the PEDro scale, and disagreements were resolved by consulting a third researcher.

3. Results

3.1. Study selection and description of the included studies

Our initial search identified 492 records (Fig. 1), and after the screening of titles, abstracts, and full texts, respectively, 13 studies were included in our final analysis. Accordingly, the characteristics of the participants, the follow-up periods, the injury definitions, and the methods used to determine the genotyping of *ACTN3* R577X (rs1815739) polymorphism are shown in Table 2. These studies were performed in 6 different countries (Spain, Japan, Brazil, China, Republic of Korea, and Italy), involving a total participant pool of 1093 participants, where 20 represents the lowest number and 257 the highest number of participants within the studies. Two studies involved only women, 5 studies involved only men, and 6 involved both men and women. All the studies included were classified as high-quality studies (≥6 in the PEDro scale score) (Table 3).

3.2. Evaluation of the effects of ACTN3 R577X genotype on non-contact injuries

The results of best evidence synthesis of the effects of *ACTN3* R577X genotype on non-contact injuries are presented in Table 4. For 12 studies,[12–15,17,18,20–22,27,29,30] evidence existed of an association between the *ACTN3* R577X genotype and non-contact injury, and for the 1 remaining study, no effect was observed.[39] From the total, 6 studies observed a significant association between the *ACTN3* R577X polymorphism and exercise-induced muscle damage,[24–26,31–33] 34 observed association between *ACTN3* R577X polymorphism and non-contact ankle injury,[21,22] and 1 observed association with non-contact sports injury (i.e., muscle strain and ligament rupture).[18] Additionally, 2 studies[17,35] observed significant associations between *ACTN3* R577X polymorphism and non-contact lower-limb injury, and 1 with non-contact musculoskeletal injury.[15] Only 1 study reported no significant association between *ACTN3* R577X polymorphism and passive stiffness or hamstring strain injury (Table 4).[19]

4. Discussion

Characterizing the influence of the *ACTN3* R577X genotype on exercise-related sport injuries is pertinent because the *ACTN3* XX genotype, which produces α-actinin-3 deficiency, has been repeatedly associated with a reduction in sports performance in sprint and power-based disciplines.[35–37] The main outcomes of this investigation indicate that most studies of the effect of *ACTN3* R577X polymorphism on injury epidemiology indicate an association of the XX genotype with a deleterious effect on injury, expressed either by increased incidence or severity, as compared to RX and RR genotypes. However, current evidence suggests that the higher likelihood of exercise-related injury in XX athletes is specific to muscle injury, ligament injury, and an exacerbation of muscle damage. It can be concluded that possessing the *ACTN3* XX genotype may predispose athletes to a higher probability of muscle and ligament injury during exercise. The evidence linking *ACTN3* genotype and injury epidemiology is still emerging, so it is premature to recommend the determination of athletes' *ACTN3* genotype for the prediction of injury risk.

4.1. Overall rate of injury

In the studies investigating all types of non-contact exercise-related injuries in athletes, the evidence indicates a higher injury incidence in RR individuals (defined as those possessing the R allele) when compared to XX individuals.[17,18] Iwao-Koizumi et al.[18] found that in female athletes training to become professional athletes, those with the R allele had a 2.52-fold higher likelihood of suffering a severe injury than did those with the X allele.[18] Similarly, Moreno et al.[17] found that among amateur runners preparing for a marathon, those with the RR genotype had a 1.79-fold higher likelihood of suffering a running-related injury when compared to their XX counterparts, whereas the likelihood of injury increased to 1.93 when comparing RR and RX athletes. Injury incidence in these marathon runners tended to be higher in RR runners; overall injury incidence was not different in phenotypes (2.78 injuries per 1000 h of running) and in RX runners (1.65 and 1.94 injuries/1000 h of running, respectively), although this difference was not statistically significant. The higher overall injury incidence in RR athletes, despite the ability to fully express α-actinin-3 in their fast-type muscle fibers,[3] may be related to other phenotypes found in RR athletes. RR athletes habitually present higher levels of muscle strength and power than their XX counterparts,[38,39] which may lead to sport-specific actions with a greater degree of speed/power.[39] In fact, this is 1 of the assumptions that may explain the higher frequency of RR athletes participating in strength-and power-based sports.[8] RR athletes may have a lower range of motion and muscle flexibility because lower-ankle dorsiflexion[7] and lower-trunk flexibility[40,41] have been found in RR *vs.* RX-XX genotypes. A greater volume or magnitude of sport-specific actions performed at higher intensity, coupled with reduced joint mobility, may explain the higher injury rates of RR athletes compared to RX-XX athletes. Nevertheless, the studies of this topic are scant, and the evidence is still not sufficient to link clearly the *ACTN3*RR genotype with a higher injury rate. Future investigations are needed to affirm whether RR individuals are more prone to overall sport-related injuries, particularly in high-intensity sports.

To summarize: When accounting for all types of injury, RR athletes may be more prone to sport-specific injury because the presence of α-actinin-3 in their muscles likely entails the production of more forceful and powerful contractions.

4.2. Muscle injury and exercise-induced muscle damage

4.2.1. Muscle injury

When investigating only non-contact muscle-type injuries, the evidence indicates that XX athletes are more prone to this specific type of injury. For example, Massidda et al.[16] found

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Table 2
Characteristics of the studies that examined the association between the *ACTN3* R577X polymorphism and the rates and severity of non-contact injuries in highly trained athletes and in individuals enrolled in exercise training programs.

Study	Country	Population/sample size/sex/age years (mean ± SD or range)/sport and level of practice	Follow-up	Injury data collection	Genetic testing
Pimenta et al. (2012)[33]	Brazil	37 male professional soccer players Age: 24.8 ± 1.7	Before and after eccentric exercise	Exercise-induced muscle damage (eccentric exercise) Post-exercise serum CK and α-actin immediately after (post), 2- and 4-h post-eccentric exercise	Extraction of genomic DNA from the peripheral blood samples. A DNA fragment carrying the exon 16 from the ACTN3 gene was amplified from the genomic DNA, and the following initiators were used: 5-CTGTTGCCTGTGGTAAGTGGG-3; reverse, 5-TGGTCACAGTATGCAGGAGGG-3, correlated to the adjacent intronic sequences.
Iwao-Koizumi et al. (2014)[18]	Japan	99 female sports students Average age: 19.7 Sports: football, softball, basketball, and badminton Level of training: future professional athletes	–	A questionnaire was developed and filled out by each participant. The questionnaire asked whether the participant had suffered severe injuries during sports activity in the past.	DNA was collected from a few drops of saliva on water-soluble paper (Mishima Dissolve Paper, 60 MDP; Nippon Paper Papylia Co., Ltd., Tokyo, Japan), Genotyping using TaqMan assay (TaqMan® SNP Genotyping Assays; Life Technologies, Carlsbad, CA, USA).
Kim et al. (2014)[21]	Republic of Korea	97 elite ballet dancers and 203 normal female adults Age: 23.1 ± 1.3	–	Structured injury questionnaire and testing for injury risks on the joints	MGB TaqMan® SNP Genotyping assay method was used to analyze ACTN3 R577X polymorphism (rs1815739) from the extracted gDNA.
Shang et al. (2015)[22]	China	142 males from a Chinese army infantry division Age: 21.0 ± 0.2	Past 1 year	Non-contact ankle sprains were diagnosed by an experienced clinician according to the standard validated criteria.	DNA was extracted from blood cells by standard procedures by the kit manufacturer (Promega, Madison, WI, USA). Genotyping was done using a TaqMan allele discrimination assay that used the 5' nuclease activity of Taq polymerase.
Belli et al. (2017)[26]	Brazil	20 men and women athletes Age: 40.5 ± 1.2	Before and after an official 37.1 km adventure race (22.1 km mountain biking, 10.9 km trekking, 4.1 km water trekking, 30 m rope course, and orienteering)	Post-exercise muscle damage	Genomic DNA was isolated from the buffy coat of centrifuged whole blood, using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Genotyping of ACTN3 R577X (rs1815739) polymorphism was conducted using a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA).
Del Coso et al. (2017)[24]	Spain	23 experienced men and women triathletes Age: 36.4 ± 5.2	Before and after the race	The changes in serum creatine kinase (CK-MM isoform) were measured in the blood samples, and muscle pain was measured with a visual analogue scale (0−10 cm).	DNA was isolated from the whole blood obtained before the race (QIAamp® DNA Blood Mini Kit, QIAGEN, The Netherlands) according to the manufacturer's protocol. Genotyping was performed using a TaqMan® SNP genotyping assay (Life Technologies™).

(*continued on next page*)

Table 2 (Continued)

Study	Country	Population/sample size/sex/age years (mean ± SD or range)/sport and level of practice	Follow-up	Injury data collection	Genetic testing
Del Coso et al. (2017)[25]	Spain	71 healthy, experienced (3 years) marathon runners, men and women Age: 42.7 ± 8.9	Before and after the race	Post-exercise muscle damage; post-exercise serum CK.	Genomic DNA was isolated from the whole blood obtained before the race (QIAamp® DNA Blood Mini Kit) according to the manufacturer's protocol. Genotyping was performed using a TaqMan® SNP genotyping assay (Life Technologies™).
Massidda et al. (2019)[16]	Italy	257 male professional soccer players Age: 19.4 ± 5.2	1−5 years follow-up	Physical complaint occurring during practice that prevented a player from participating in training or match play for at least 1 day after the day of the onset. Ultrasound and magnetic resonance imaging scans were used to morphologically classify the injuries.	DNA was extracted from a buccal swab and a commercially available kit (QIAGEN, Hilden, Germany) was used. Concentration of extracted DNA was determined by fluorometric method (through Qubit by Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA).
Miyamoto et al (2018)[42]	Japan	76 male sports university students Age: 21.2 ± 2.8 Sport: multiple sports Level of training: regular training/recreationally active	–	Past hamstring-muscle strain injury	DNA was extracted from saliva and collected with a DNA self-collection kit (Oragene, DNA Genotek, Ontario, Canada) according to the manufacturer's protocol. DNA was quantified using a spectrophotometer (Eppendorf Bio Photometer Plus, Eppendorf, Tokyo, Japan).
Del Coso et al. (2017)[31]	Spain	67 healthy and experienced marathon runners (male, all Spanish Caucasians) (low CK: 36, age: 43.0 ± 8.1, high CK: 31, age: 41.4 ± 9.4)	Immediately after the race	Post-exercise muscle damage	A DNA fragment carrying the exon 16 from the ACTN3 gene was amplified from the genomic DNA and the following initiators were used: 5-CTGTTGCCTGTGGTAAGTGGG-3; reverse, 5-TGGTCACAGTATGCAGGAGGG-3, correlated to the adjacent intronic sequences.
Clos et al. (2019)[15]	Spain	43 male professional soccer players. Age: 27.5 ± 1.2	7 years follow-up	Injury rate, injury severity, and injury recovery times were established. Injury severity was established according to the days a player needed to be absent from training and competition: mild, 1−15 days; moderate, 16−30 days; severe, more than 30 days.	Genomic DNA isolation was performed using a QIAmp DNA Blood Minikit (Qiagen, Valencia, CA, USA). DNA quantity was measured with a Nano-Drop ND-1000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

(*continued on next page*)

Table 2 (Continued)

Study	Country	Population/sample size/sex/age years (mean ± SD or range)/sport and level of practice	Follow-up	Injury data collection	Genetic testing
Del Coso et al. (2020)[34]	Spain	22 experienced men and women triathletes Age: 35.4 ± 4.3 Low CK, *n* = 10 High CK, *n* = 12	Before and after the race	Post-exercise muscle damage	A DNA fragment carrying the exon 16 from the ACTN3 gene was amplified from the genomic DNA and the following initiators were used: 5-CTGTTGCCTGTGGTAAGTGGG-3; reverse, 5-TGGTCACAGTATGCAG-GAGGG-3, correlated to the adjacent intronic sequences.
Moreno et al. (2020)[17]	Spain	139 (XX: 32, RX: 67, RR: 40) Healthy marathon runners, men and women Age: 41.3 ± 10.2	1 year preceding to marathon	Physical complaints/visible damage to any part of lower limb assessed by qualified medical/healthcare practitioner	DNA was isolated using an organic-based DNA extraction method adapted to Amicon1 Ultra 0.5-mL columns, including a final concentration step to 50 μL.

Abbreviations: CK = creatine-kinase.

Table 3
Physiotherapy evidence database (PEDro) score of some long-term studies.

	PEDro items											
Study	Eligibility criteria	Random-allocation	Concealed-allocation	Group homogeneity	Blinded subjects	Blinded therapists	Blinded assessor	Drop out 5%	Intention to treat analyses	Between-group comparison	Point estimates and variability	Score
Pimenta et al. (2012)[33]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Iwao-Koizumi et al. (2014)[18]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Kim et al. (2014)[21]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Shang et al. (2015)[22]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Belli et al. (2017)[26]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Del Coso et al. (2017)[24]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Del Coso et al. (2017)[25]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Del Coso et al. (2017)[31]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Massidda et al. (2019)[16]	•	◯	•	•	•	◯	•	◯	◯	•	•	6
Miyamoto et al. (2018)[42]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Del Coso et al. (2020)[34]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Clos et al. (2019)[15]	•	◯	•	•	•	◯	•	◯	◯	•	•	7
Moreno et al. (2020)[17]	•	◯	•	•	•	◯	•	◯	◯	•	•	7

Table 4
Results of best evidence synthesis of association between *ACTN3* R577X genotype and non-contact injuries.

Studies	Main study outcomes	Best evidence synthesis	
		Presence of association	Level of evidence
Pimenta et al. (2012)[33]	*ACTN3* R577X polymorphism and muscle damage After eccentric training, XX athletes presented higher levels for CK (4-h post) and α-actin (post and 2-h post) compared to RR and RX. XX athletes were more susceptible to muscle damage after eccentric exercise compared to RR and RX athletes.	Yes	High
Iwao-Koizumi et al. (2014)[18]	*ACTN3* R577X polymorphism and non-contact sports injury RR genotype and R allele frequency was higher than XX frequency in athletes who experienced muscle injury.	Yes	High
Kim et al. (2014)[21]	*ACTN3* R577X polymorphism and injury risk *ACTN3* polymorphism was associated with ballerinas' performance capacity. Injury frequency in the ankle in XX ballerinas was 4.65 times higher than in RR and RX counterparts.	Yes	High
Shang et al. (2015)[22]	*ACTN3* R577X polymorphism and non-contact ankle sprain The frequency of the XX genotype was significantly higher among the acute ankle sprains group than the control group (52.1% *vs*. 41.1%).	Yes	High
Belli et al. (2017)[26]	*ACTN3* R577X polymorphism and muscle damage Athletes with the XX genotype presented a higher magnitude of muscle damage than RX and RR after an adventure race.	Yes	High
Del Coso et al. (2017)[24]	*ACTN3* R577X polymorphism and exercise induced muscle damage. X-allele triathletes presented greater signs of exercise-induced muscle damage during a half-ironman race than RR homozygotes. XX and RX (X-allele group) presented higher self-reported values of lower limb muscle pain than RR.	Yes	High
Del Coso et al. (2017)[25]	*ACTN3* R577X polymorphism and exercise induced muscle damage. In comparison to RR homozygotes, X allele carriers for the R577X polymorphism of the ACTN3 gene presented higher values for typical markers of exercise-induced muscle damage during a competitive marathon.	Yes	High
Massidda et al. (2019)[16]	*ACTN*3 R577X polymorphism and non-contact lower limb injury The ACTN3 R577X polymorphism is associated with the incidence and severity of muscle injuries in professional football players; players with the ACTN3 577XX genotype have higher odds of having muscle injuries than their RR counterparts.	Yes	High
Miyamoto et al. (2018)[42]	*ACTN3* R577X polymorphism and passive stiffness and hamstring strain injury NS difference between genotypes for the frequency of past hamstring strain injury ($p = 0.587$)	No	High
Del Coso et al. (2017)[31]	*ACTN3* R577X polymorphism and exercise induced muscle damage. Marathoners with a lower CK response after a marathon had a more favorable polygenic profile than marathoners with high serum CK concentrations.	Yes	High
Clos et al. (2019)[15]	*ACTN3* R577X polymorphism and non-contact musculoskeletal injury NS difference between genotypes for non-contact musculoskeletal soft-tissue injury rate XX are the ones with the higher injury rate.	Yes	High
Del Coso et al. (2020)[34]	*ACTN3* R577X polymorphism and exercise induced muscle damage. Total genotype score was higher in low-CK responders than in high-CK responders. A favorable polygenic profile can contribute to reducing the level of muscle damage developed during endurance exercise.	Yes	High
Moreno et al. (2020)[17]	*ACTN3* R577X polymorphism and non-contact lower limb injury XX runners had a higher frequency of sudden injuries ($p = 0.024$) and the OR for muscle-type injury was 2.0 (0.51−7.79) times higher than RR.	Yes	High

Abbreviations: NS = not significant; OR = odds ratio.

that, among male professional football players, the odds were 2.66 times higher for those with the XX genotype to suffer a muscle injury than for their RR counterparts, with no difference between RR and RX football players. Additionally, the XX players had a 2.13-fold higher probability of having a severe injury compared with RR players, despite similar training exposure among genotypes. Similar results were shown by Clos et al.[15] in a sample of professional football players; those

with the XX genotype presented a 1.84-fold greater likelihood of suffering a muscle-type injury, although there was no difference in severity.[15] In a sample of amateur marathoners, Moreno et al.[17] showed that XX runners presented a 2.0-fold greater likelihood of muscle-type injuries when compared to RR runners. This was evident only for muscle-type injuries because the probability of the overall injury (i.e., including all types of injuries) was higher in RR *vs.* XX individuals. On the other hand, Miyamoto et al.[42] did not find any effect of the *ACTN3* genotype on the frequency of hamstring strain injuries in a sample of young male athletes. However, this investigation was based only on a specific body location, and that could have reduced the likelihood of finding differences among the genotypes. Collectively, these investigations indicate that α-actinin-3 deficiency, due to homozygosity in the ACTN3 R577X polymorphism, may entail a higher probability of non-contact muscle injuries. Del Coso et al.[7,10] proposed that this could be explained by the lower capacity of α-actinin-3-deficient muscle fibers to endure physical stress. Interestingly, α-actinin-3 deficiency is habitually compensated for by a higher amount of α-actinin-2.[43] The current data concerning differing muscle-injury incidence among genotypes indirectly suggests that the role of α-actinin-3 is different from the role of α-actinin-2, which may explain why these muscle sarcomeric isoforms of α-actinin have remained through evolution; however, further work to elucidate the veracity of this claim is warranted.

4.2.2. Exercise-induced muscle damage

Additional evidence suggesting that α-actinin-3 deficiency, due to the *ACTN3* XX genotype, results in muscle fibers with less capacity to endure stress during exercise was found in those investigations that assessed muscle damage. When compared to RR counterparts, carriers of the *ACTN3* X allele presented higher values of indirect markers of muscle damage, such as (1) reduced jump height[24,25]; (2) postexercise or pre- to postexercise changes in serum creatine kinase or myoglobin concentrations[33,39]; and (3) self-reported muscle-pain values.[25] The results are similar when comparing XX athletes to those with the R allele,[26] suggesting that the R allele may have a dose-response effect, playing a protective role to prevent exercise-induced muscle damage (i.e., RR > RX > XX). Higher markers of exercise-induced muscle damage in XX individuals have also been found in some non-athletic populations,[44] although this is not always the case.[45] These outcomes indicate that α-actinin-3 has a positive role in modulating the capacity of muscle to endure potentially damaging contractions during exercise,[37] whereas α-actinin-3 deficiency due to homozygosity in the *ACTN3* R577X polymorphism may entail a higher probability of muscle injury/muscle damage during exercise. Of note, *ACTN3* is 1 of several genes involved in the response to exercise-induced muscle damage; the capacity of skeletal muscle to endure the physical stress imposed by exercise may be associated with possessing favorable genotypes in several genes.[24,32]

To summarize: When accounting for muscle-specific injury and exercise-induced muscle damage, α-actinin-3 deficiency resulting from homozygosity in the null-X allele of the *ACTN3* gene was associated with a higher incidence of injury as well as with injuries of higher severity.

4.3. Ligament injury

In the current review, 2 independent investigations determined that XX individuals are more prone to ligament injury, specifically in the ankle.[21,22] According to the odds ratio analysis performed by Kim et al.,[21] elite ballerinas with the XX genotype had a 4.7-fold higher risk of ankle injury than the RR and RX-genotyped ballerinas, although this greater risk of injury was not present in other body locations, such as the knee, pelvis, or back. In an investigation done by Shang et al.,[22] the frequency of the XX genotype in lance corporals with ankle sprain was higher than that in a control group without this type of injury. Interestingly, this investigation revealed that the XX genotype is associated not only with the likelihood of injury but also with the severity; the presence of the X allele resulted in greater gravity of an injury (e.g., ankle sprains from grade I to grade III). As previously suggested,[36] the higher likelihood of ligament injury in α-actinin-3-deficient athletes might be related to a dysfunction in the capacity of the muscle to hold the joint during sport-specific actions rather than to an effect of the XX genotype on the characteristics of the ligament. This is because, to date, there is no evidence suggesting that *ACTN3* R577X polymorphism has any impact on ligament tissue. XX athletes may be more prone to ligament injury during exercise, although this evidence exists for the ankle joint only.

To summarize: Further replication investigations are needed to confirm this finding and to expand the perspectives to other joints.

5. Conclusion

The current systematic review indicates that most studies of the effect of the *ACTN3* gene relative to injury epidemiology in athletes have found an association between the *ACTN3* genotype and injury risk and severity. Specifically, possessing the *ACTN3* XX genotype may predispose athletes to a higher probability of some non-contact injuries, such as muscle injury, ankle sprains, and higher levels of exercise-induced muscular damage. Notwithstanding this interesting finding, we acknowledge that this conclusion is based on a limited number of studies (n = 13). Evidence is still emerging and, thus far, has been obtained from studies with very heterogeneous samples of athletes, with different methodologies used to quantify injury incidence and epidemiology and, in most cases, with low or small samples sizes, all of which can complicate interpretations of data.[46] Despite the preliminary evidence for the notion that the XX genotype might be associated with a higher risk of injury issues, the common view is that there is no current clinical application for genetic testing in the area of exercise prescription and injury prevention.[47,48] The evidence herein suggests that this may not be the case. Future research into this topic is warranted in order to produce more convincing evidence-based approaches to either support or refute *ACTN3* genotyping for injury prevention.

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Authors' contributions

HZ and JDC were involved in the conceptualization of the study, data analysis, and the writing of the manuscript; AJ, CT, GR, NJ, CCTC, BB, and ABA were involved in the data assessment, data analysis, and the writing of the manuscript; ACH was involved in the writing of the manuscript. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jshs.2021.07.003.

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